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FLUID AND PROTEIN SHIFT WITH HEMORRHAGE AND WITH OVERTRANSFUSION

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ABSTRACT

1. Preparing Institution: Baylor University College of Medicine

2. Title of Report: The Circulatory Effects of a Homologous Plasma-Blood Exchange in the Dog.

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The effect on plasma and red cell volume and protein concentration of exchanging homologous plasma for the dogs' own blood before a hemorrhage to 35 mm Hg mean arterial pressure was tested in morphine-pentobarbitalized dogs. The exchange was accomplished by bleeding the animals from an artery (44 cc/kg of blood) and at the same rate simultaneously infusing homologous plasma (44 cc/kg) through a vein. The measured cell and plasma volumes and protein concentration were in good agreement with the expected after the exchange. Therefore, homologous plasma successfully replaced the dogs' plasma removed during the exchange, and no reaction was seen attributable to the plasma infusion. When these animals were subjected to hemorrhage, the mean bleeding volume was 30% to reach a level of 35 mm Hg arterial pressure in contrast to 46% for dogs not receiving an exchange. Also 5.9 cc/kg of plasma and 0.46 gm/kg of protein has escaped from the circulation while previously there was mobilization of fluid and protein after a hemorrhage.

The Circulatory Effects of a Homologous Plasma-Blood Exchange in the Dog

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Submitted for publicotion.

Recently data has been presented indicating that homologous plasma or blood differs from autologous by producing a certain group of responses when infused into a dog. Bliss et.al. (1), first reported that intradermal injection of plasma from another dog produced well marked wheals. Then, reasoning that such a marked intradermal response could be paralleled by a systemic response if homologous plasma was infused, an experiment was done to investigate this possibility on normovolemic dogs. On infusion of homologous plasma, urticaria developed and the dogs did not retain the infused fluid and protein within the circulation. These phenomena did not occur if autologous plasma was infused (2).

These observations were confirmed and extended further by investigations which described the following symptoms occurring with transfusion of homologous blood or plasma into normovolemic dogs: fall in blood pressure, skin edema, labored respiration, prolonged bleeding time and death of a majority of dogs within a 24 hour period. Associated with this syndrome, and presumably a part of it, was a descrepancy between the measured and expected plasma volume: the measured plasma volume was always less than the expected. None of these reactions occurred when autologous blood or plasma was infused (3).

These various responses were suggestive of a foreign protein reaction and

because they occurred with the infusion of homologous plasma as well as blood, it was suggested that an unknown "plasma factor" must be the inciting agent (1-3).

The problem of hypervolemia resulting from such transfusions was avoided by Lawson et.al. (4) by simultaneously withdrawing a volume of blood equivalent to that infused. Under these circumstances the distribution spaces of the dye T-1824 and P^{32} agreed with those calculated from the dilution of the tags when untagged autologous whole blood but not homologous was used for the exchange. In another experiment in which autologous blood was transfused to increase the blood volume of normovolemic dogs, the agreement for the cell volume was good, but large differences occurred between the plasma volumes determined initially by T-1824 and that calculated subsequently from the dilution of the tag.

Contrary to these data when dogs were rendered hypovolemic, plasma volume restoration occurred equally well whether homologous or autologous plasma was used. Further, there were only 2 of the 19 dogs that developed wheals, and these were minor in character (5).

To test the reported difference between the measured and expected plasma volumes when homologous blood or plasma was infused, an experiment was devised using dogs in which homologous plasma was exchanged for blood so that the blood volume was not expanded.

METHODS

Seven dogs were anesthetized with 10 mg/kg of morphine sulfate subcutaneously followed by 15 mg/kg of sodium pentobarbital in 20 to 30 minutes. The routine procedures used in this laboratory for this type of experiment are discussed in detail in another paper (6). Cell and plasma volumes were determined by Cr^{51} tagged red cell and T-1824 dye dilution methods, respectively (7,8). Specific gravity of the plasma proteins was determined on all samples by the falling drop method (9), and from these data the protein concentration was calculated. The hematocrit was measured in Wintrobe blood index tubes centrifuged at 2,500 rpm for 20 minutes and corrected by 0.95 for the trapped plasma. Blood samples were taken by catheter from the right atrium at 10, 20 and 30 minutes after injection of the tagging materials. Carotid arterial pressure was measured with a mercury manometer. After the samples for the control determinations had been drawn, the dogs were bled from the left femoral artery and plasma from a donor dog was infused simultaneously through the right femoral vein. The rate of the exchange was regulated in such a way that the rate of blood removed was the same as the rate of plasma infused, thus blood loss equalled the plasma gained. The volume of the exchange varied from 40 to 50 cc/kg with a mean volume of 44.0 cc/kg. Cell and plasma volume, hematocrit and protein determinations were made after

the blood plasma exchange. Samples were taken at 10, 20, 30 and 60 minutes after the dye and tagged red cell injections. Then the tube leading to the bleeding bottle was reopened and the dogs were bled slowly to a mean arterial pressure of 35 mm Hg. When the mean arterial pressure of the dog fell below 35 mm Hg, the tube was clamped and the final measurements were made.

RESULTS

A mean volume of 44.0 cc/kg of blood was removed and 44.0 cc/kg of plasma was simultaneously infused. The exchange was accomplished with only a small decrease in mean arterial pressure from the control level of 101 to 91 mm Hg. However, one dog, not included in the mean value, had a precipitous fall in pressure. After the exchange the agreement between the measured and expected plasma and cell volume and protein concentration was very good. As a result of the exchange of plasma for blood, the venous hematocrit fell from 42.4 to 23.9%, the circulatory hematocrit from 36.8 to 19.3%, and the BVR_{cells} decreased from 0.87 to 0.80 (Table 1).

Then the dogs were bled slowly to a mean arterial blood pressure of 35 mm Hg. The mean bleeding volume to reach this pressure was 23 cc/kg, and represented 30% of the measured blood volume. Contrary to our usual experience, the blood pressure tended to fall progressively during the final determinations. The

blood volume after the hemorrhage was significantly less than the expected because 5.9 cc/kg of plasma apparently left the circulation. At the same time there was a loss of 0.46 gm/kg of protein reducing the concentration from an expected 5.25 to a measured 5.08 gm/100cc. The concentration of protein leaving the circulation was 7.8 gm/100cc of plasma.

DISCUSSION

Depending on the experiment, the infusion of homologous plasma or blood results in effects different than those obtained with autologous plasma or blood (2-5). The present experiment, with some modifications, is similar to one reported by Lawson et.al. (4), in which they utilized the principle of exchanging the blood of a dog for either homologous or autologous blood or plasma. However, the plasma used for the autologous experiment was contaminated, as they point out, by as much as 50% homologous plasma. In spite of this, they found that with this so-called "autologous" plasma infusion the distribution space of T-1824 agreed with the space calculated from the infused unlabeled plasma. This agreement did not occur if only homologous blood or plasma was infused. However, when autologous plasma was used to expand the blood volume of a normovolemic dog the results were more like those obtained with the exchange experiment in which homologous blood or plasma was used; although this experiment, too, was

complicated by the use of homologous blood to replace autologous blood in order to maintain the original blood volume.

In our initial experiment, which was comparable to their exchange experiment with homologous plasma, both the measured cell and plasma volumes agreed well with the expected volumes. The latter was calculated from the initial measurements and the red cells and plasma removed from and added to the circulation with the exchange. Further, none of the dogs developed reactions attributed to homologous blood or plasma by various investigators, although it has been reported previously that about 10% of the dogs overtransfused with homologous blood developed a mild urticaria (10). Therefore, it was found that homologous plasma successfully replaced the dogs' plasma removed during the exchange, and it can be concluded that under these experimental conditions homologous plasma does not produce any effect other than would have been expected with autologous plasma.

However, there were striking differences between these dogs subjected to a blood-homoiogous plasma exchange and control dogs subjected to hemorrhage. From previous data, for example, when dogs were bled to 35 mm Hg the mean bleeding volume was 46% of the initial blood volume, and 7 cc/kg of fluid was added to the circulation with a mean protein content of 3.2 gm/100cc (11). The dogs in the present experiment, on the contrary, only bled a mean of 30% of their blood volume to reach an arterial pressure level of 35 mm Hg. The plasma volume and total protein, instead of being greater than the expected, was less than the

expected because 5.9 cc/kg of plasma and 0.46 gm/kg of protein has left the circulation.

These data lead to the conclusion that the exchange of homologous plasma for the animal's blood before the hemorrhage has in some manner, at present unknown, both reduced the bleeding volume and prevented the usual movement of fluid and protein into the circulation accompanying a hemorrhage to 35 mm Hg arterial pressure. There is no evidence indicating that the same results might not have been obtained if autologous plasma had been used for the exchange. Unfortunately, neither the present experiments nor those of Lawson et.al. (4) permit even tentative conclusions concerning the mechanism producing this effect.

The hematocrit of the blood removed during hemorrhage was the same as the venous hematocrit and higher than the circulatory hematocrit, consequently, in spite of plasma leaving the circulation with hemorrhage the latter did not increase, but on the other hand the venous hematocrit decreased after hemorrhage. Thus, it may be assumed that plasma from the small vessels or low hematocrit compartment entered the large vessel system or high hematocrit compartment (12). Consequently, the BVR_{cells} increased from 0.80 after the plasma-blood exchange to 0.91 after the hemorrhage.

TABLE I. Plasma-blood exchange followed by hemorrhage to 35 mm Hg mean arterial pressure.

	Venous Hematocrit	Plasma Volume	Cell Volume	Blood Volume	Circulatory Hematocrit	BVR_{cells}	Plasma Proteins	
	%	cc/kg	cc/kg	cc/kg	%		gm/100cc	gm/kg
Control	42.4 ± 3.36*	54.2 ± 6.84	29.6 ± 1.59	83.7 ± 6.44	36.8 ± 3.68	0.87 ± 0.05	5.51 ± 0.24	2.90 ± 0.30
Blood lost †	31.0 ± 2.44	33.5 ± 2.00	15.0 ± 0.87				5.55 ± 0.19	1.86 ± 0.10
Plasma infused		44.0 ± 1.62					5.48 ± 0.28	2.42 ± 0.16
Expected after exchange		64.8 ± 5.59	14.6 ± 1.50	79.4 ± 5.80	18.7 ± 2.15		5.43 ± 0.24	3.46 ± 0.26
Measured after exchange	23.9 ± 1.99	63.0 ± 5.80	14.6 ± 1.39	77.6 ± 6.73	19.3 ± 2.80	0.80 ± 0.05	5.49 ± 0.21	3.42 ± 0.29
Hemorrhage blood	23.9 ± 2.24	17.5 ± 2.46	5.5 ± 1.28	23.0 ± 3.57			5.60 ± 0.35	0.98 ± 0.11
Expected after hemorrhage		45.5 ± 5.89	9.1 ± 0.91	54.6 ± 6.37	18.5 ± 3.46		5.25 ± 0.26	2.44 ± 0.35
Measured after hemorrhage	21.2 ± 1.58	39.6 ± 5.30	8.8 ± 0.47	48.4 ± 5.26	19.8 ± 3.24	0.91 ± 0.09	5.08 ± 0.27	1.98 ± 0.24

*Mean values for 7 dogs with a mean weight of 11.4 ± 0.38 kg.

† These values include the samples withdrawn.

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In addition, work accomplished in this laboratory since the beginning of contract number DA-49-193-MD-2357 is summarized by the following abstracts of papers submitted for publication to the American Journal of Physiology.

I. Volume Distribution of Evans Blue Dye and Iodinated Albumin in the Dog.

R. A. Huggins, E. L. Smith and S. Deavers

372 morphine-pentobarbitalized dogs were divided into groups according to the sampling time after injections of T-1824, I^{131}, or both simultaneously. The data were programmed for digital computer analysis. In dogs receiving the tags separately the mean plasma volume was 50.0 $\pm$ 0.63 and 49.8 $\pm$ 0.72 cc/kg for dye and I^{131}, respectively. Three simultaneous T-1824 and I^{131} tagged albumin injections were made with samples taken at 5, 10, 15, 20, 30, 45 and 60 minutes after each injection. Mixing was complete at approximately 5 minutes. Mean plasma volumes were 52.5 $\pm$ 2.24 cc/kg for the dye and 49.3 $\pm$ 1.69 cc/kg for the I^{131}. The difference was not significant. In this group there was a good agreement between plasma volumes when calculated from the 7 point curve or from 3 points only, indicating that all points were part of the same straight line. Therefore, a shorter time period with fewer samples is advantageous. Furthermore, when maximum accuracy is not required, the 5 minute sample gives a reasonable estimate of plasma volume. There was an apparent steepening of the disappearance slope with the third injections of both dye and I^{131}.

Accepted for publication; Am. J. Physiology, 1963.

II. The Movement of Fluid, Albumin and Globulins With Overtransfusion and Hemorrhage.

S. Deavers, E. L. Smith and R. A. Huggins

Two groups of 18 morphine-pentobarbitalized dogs were used for the experiments. Determinations of plasma volume and protein concentration were made before and after the experimental procedures. The first group was bled to a mean arterial pressure of 35 mm Hg. During an average bleeding time of 74 minutes in which the dogs bled 48% of their circulating plasma and 47% of their total protein, 29% of the plasma and 14% of the protein removed has been replaced from extravascular sources. Those dogs that bled for the longest time gained the greatest amount of fluid and protein. Globulins comprised 74% of the protein mobilized. The second group of dogs was overtransfused with a mean volume of 85 cc/kg of whole blood. Thirty minutes after the transfusion, 20 cc/kg of plasma and 0.53 gm/kg of protein escaped from the vascular system. No correlation was found between the rate of infusion and the rate of fluid and protein loss. Seventy percent of the total protein leaving the circulation was albumin.

Submitted for publication; Am. J. Physiology, 1963.

III. The Effects of Histamine on the Distribution of Cells and Plasma in Dogs.

E. L. Smith, S. Deavers and R. A. Huggins

Cell, plasma volumes, protein concentration and venous hematocrit were measured on 10 morphine-pentobarbitalized dogs before and after subcutaneous histamine diphosphate (1 mg/kg base). The mean arterial pressure fell from 98 to 50 mm Hg. Cell and plasma volumes and the venous hematocrit increased while the protein

concentration decreased after histamine. The circulatory hematocrit did not change, consequently the BVR_{cells} decreased from 0.88 to 0.78 after histamine. Assuming that the circulation can be divided into a low and high hematocrit compartment. the volume of these compartments can be calculated; they contained 33 and 67% of the measured blood volume, respectively. Although the compartments contained the same percentage of blood after histamine, there was a shift of cells from the low to the high hematocrit compartment while plasma shifted in the reverse direction, resulting in a higher venous hematocrit. A good agreement between the percentage of measured cell and plasma volumes in the tissues of control dogs and in the calculated low hematocrit compartment lend support to the present calculations.

Submitted for publication.

IV. Disappearance Rates of Multiple Injections of T-1824 and I^{131} Tagged Albumin.

S. Deavers, E. L. Smith and R. A. Huggins

Sixteen morphine-pentobarbitalized dogs were given T-1824 dye three or four times one hour apart. Radioactive tagged albumin was administered simultaneously with the last dye injection. Another sixteen dogs received the tags in the reverse order. With successive injections, the rate of dye disappearance was the same as for a single injection; approximately 10% in 60 minutes and iodinated albumin left the circulation at the same rate as the dye. When the iodinated albumin was injected three or four times and the dye given with the last injection, there was a slight but not significant difference between their rate of disappear-

ance. When the background from previous injections was subtracted before the line of best fit and intercept ("zero" minute concentration) were calculated, the disappearance slope of the last injection steepened significantly. This was an arithmetical artifact and had no physiological validity. When five times the usual amount of dye and I^{131} tagged albumin was administered simultaneously, their disappearance rates were again 10% during one hour. The mean plasma volumes were in good agreement for either dye or iodinated albumin.

Submitted for publication; Am. J. Physiology, 1963.

Meetings attended and papers presented:

Southwestern Section of the Society for Experimental Biology and Medicine, March 9-10, 1963, Galveston, Texas.
The Effect of Histamine on the Distribution of Cells and Plasma in Dogs.
S. Deavers, R. A. Huggins and E. L. Smith

Federation of American Societies for Experimental Biology, April 16-20, 1963, Atlantic City, New Jersey.
Effects of Multiple Injections of T-1824 Dye and Iodinated Albumin on Plasma Volume Determinations.
S. Deavers and R. A. Huggins